JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2014. Vol. **8**(6), p. 4647-4656

Evaluation of Antifungal Activity of Lactic Acid Bacteria (LAB) and Plant Products against Human Pathogenic and Food Spoilage Fungi

Payal Choudhary and V. Devi Rajeswari*

School of Biosciences and Technology, Vellore Institute of Technology, Vellore-14, India.

(Received: 16 July 2014; accepted: 20 August 2014)

Present research was aimed at investigating the control of food spoilage fungi by lactic acid bacteria and natural plant extracts. In this experiment a total of twelve bacterial isolates were aseptically isolated from four different food samples such as lemon, buffalo's milk, yakult and sporlac by using serial dilution agar plate technique. At the same time a total of four spoilage fungi was isolated from different spoiled food samples such as spoiled namkeen, tomato and bread namely *Aspergillus, Fusarium, Penicillium and Alternaria* species. Along with the extracted food spoilage fungi we even used *Candida albicans* culture which is a human pathogenic fungus to testify our results. Agar well diffusion method was followed for evaluating the antifungal activity of lactic acid bacteria and plant extract against the isolated food spoilage fungi. After this we tested the antifungal susceptibility patterns of food spoilage fungi against four antifungal discs namely Clotrimazole CC ^{10}, Ketoconazole KT ^{50}, Miconazole MIC^{30} and Nystatin NS ^{50}.The minimum inhibitory concentration (MIC) of three different plant extract that had shown inhibitory effect against the food spoilage fungi was determined against the food spoilage fungi. To conclude the best antifungal effect against the isolated food spoilage fungi was shown by black pepper extract.

Key words: Food spoilage, Lactic acid Bacteria, Antifungal.

Food is any substance when consumed provides nutritional support to the body of the organism. And when this substance is ingested and assimilated by the organism's cells it produces energy in order to maintain life and stimulate growth[1]. Food spoilage is a metabolic process that causes the food to be undesirable or unacceptable for human consumption due to the influence of moisture, heat, light, and air, which in turn fosters the growth of microorganisms in food[2]. Chemical preservatives contain antimicrobial compounds that when added to food prevent spoilage of food and enhances its shelf life. But these chemical preservatives are not readily accepted by the consumer as they are toxic in nature as well as some fungi develops resistant against them after continuous use. So to avoid this situation we use an alternative method called as bio-preservatives where the antimicrobial compounds are extracted from the nature. This helps in the extension of the shelf life and food safety by the use of controlled and natural microbiota.

Pathogenic spoilage micro organisms causes serious intoxication in the food without causing any change in the color, texture, smell, appearance or taste which makes it difficult to recognize if the product is contaminated[3]. Whereas when the non-pathogenic microbes act on the

* To whom all correspondence should be addressed.
E-mail: sdevirajeswari@gmail.com

food, they cause the loss of smell, appearance taste or texture of the food but such food is still safe to consume[4]. The food spoilage microbes influence the quality, availability, and quantity of the food. The range of microorganisms associated with the spoilage of the food includes bacteria, fungi, molds, yeast and virus (Fig. 1).

The rate of food spoilage is affected by various factors which are broadly classified into two, intrinsic factors and extrinsic factors[1]. Intrinsic factors are those which exist as a part of food product itself. These include the nutrients content, pH, water activity, and structure of the food. Extrinsic factors are related to the surroundings. These include the temperature, oxygen, handling procedures[5,6].

The preservation of the food involves the addition of certain antimicrobial compounds. These antimicrobial compounds may be chemical preservatives and the process of preservation is known as chemical preservation. Chemical preservation is achieved by the addition of certain antimicrobial agents which inhibit the growth of undesirable microorganisms. These microbial agents known as chemical preservatives are added into the food to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes and thus increasing its shelf life[7].Thus an alternative way to the chemical preservation is the BIOPRESERVATION. Bio-preservation can be defined as the extension of the shelf life and food safety by the use of natural or controlled microbiota and / or their antimicrobial compounds[8].The biopreservation of the food can be done by the use of lactic acid bacteria and plant products.

Lactic acid bacteria (LAB) are Gram positive, microaerophilic, either rod or coccus, catalase negative, non spore forming bacteria that produce lactic acid as the main product from sugar fermentation .Lactic acid bacteria produce a wide range of antimicrobial compounds such as organic acid, hydrogen peroxide, and bacteriocin like molecules, formic acid, fatty acids, reuterin, reutericylin and other enzymes of importance[9].

Bio-preservation can also be done by using plant products. Their use in the food products are gaining a lot of interest due to the rich historical background, moreover these products are natural thus intended safe for use[10].A wide range of antimicrobial agents have been described from various plants. The plants used in the present study includes pomegranates (Punica granatum L), black pepper and turmeric (Curcuma species) etc which produces one and more antimicrobial compounds mentioned above. The chemical preservatives used to preserve the food have harmful effects on human health moreover some microbes had developed resistance against these chemical preservatives. Thus keeping in mind these problems, the need of the hour is to search a novel compound from plants and / or lactic acid bacteria that can be used to increase the shelf life of the food. Hence the present work aimed at the following objectives:-

a) Isolation of lactic acid bacteria from different food samples.
b) Isolation of food spoilage fungi from different plant products
c) Screening of antifungal activity of lactic acid bacteria and plant extract against isolated food spoilage fungi
d) Antifungal susceptibility pattern of tested food spoilage fungi
e) MIC of plant extract against food spoilage fungi

MATERIALS AND METHODS

Isolation of lactic acid bacteria from food samples

A total of 4 samples were collected for the isolation of Lactic Acid Bacteria from the fields and markets of Ambala and Kurukshetra, Haryana (Table 1) Serial dilution agar plate technique using MRS broth that was purchased from HiMedia Laboratories Pvt. Ltd. Mumbai India was used for isolation of lactic acid bacteria[11, 12, 13]. The isolates which were anaerobically produced were sub-cultured and purified 3 times on the MRS agar plates. Purified strains of LAB were preserved in MRS broth using 15% (v/v) glycerol at 4ºC [14].

List of food samples collected for isolation of lactic acid bacteria (table 1)

Preliminary identification of lactic acid bacteria

Three tests such as Gram staining, Endospore staining[15] and Catalase test[16] were performed for the preliminary identification of lactic acid bacteria.

Isolation of food spoilage fungi

Spoiled food samples were used for the isolation of food spoilage fungi. A total of four

spoiled food sample namely namkeen, bread and tomato (Table 2) were collected and brought to the laboratory for the isolation of food spoilage fungi. Sterile inoculation loop was rubbed over the infected/spoiled part of the sample and was streaked wove the Potato Dextrose Agar (PDA) plates. The plates were incubated at 25ºC for 3-5 days using inoculation method. The pure isolates were maintained in PDA slants at 4ºC[17].

Identification of the isolated fungi

The fungal isolates were identified on the basis of colony characteristics (i.e. color, exudates production, growth of the colony from the top and the bottom view) and sporangial structures (conidial head, types of conidiogenous cells, arrangements of conidia, sporangial head, type of spores) using lacto phenol cotton blue method[11, 12].

Production of antimicrobial metabolite from lactic acid bacteria

The hundred ml of autoclaved MRS broth was inoculated with the LAB isolates under sterile conditions and incubated at 37ºC for 24-48 hours anaerobically. The turbid medium containing the antimicrobial metabolite was centrifuged at 1000 rpm for 15 minutes to remove the cells. The pellet was discarded and supernatant was used to determine the antifungal activity[17].

Screening of antifungal activity of lactic acid bacterial metabolite:

The cell free supernatant (CFS) was screened for the antifungal activity against the isolated food spoilage fungi by agar well diffusion method[17].

Evaluation of antifungal activity of plant products:

Sample collection

Total of three samples namely pomegranate, black pepper and turmeric were collected from the market of Ambala, Haryana and brought to the laboratory for the preparation of plant extracts (Table 3). The plant parts such as peel and seeds were washed thoroughly with distilled water. The samples were dried in oven at 50ºC overnight. The dried materials were crushed into the powder in the pestle and mortar. Solvent selection: The three solvents namely ethanol, methanol and acetone were selected for the preparation of plant extracts[18]. The antifungal activity of plant extracts was determined by agar well diffusion method[19].

Collection of *Candida albicans* culture

Our sample was collected from the Department of Microbiology, Kurukshetra University.

Antifungal susceptibility testing of food spoilage fungi

For antifungal susceptibility testing, four antibiotics namely Clotrimazole, Ketoconazole, Miconazole and Nystatin were used (Table 4) using agar well diffusion method[19].

Determination of minimum inhibitory concentration (MIC) of plant extracts against the isolated food spoilage fungi

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of microorganism after overnight incubation. MIC of plant extracts were determined by macro dilution agar method[11, 12].

RESULTS

Isolation of lactic acid bacteria from food samples

Lactic acid bacteria were isolated from four different food samples such as lemon, sporlac, Yakult (probiotic drink) and buffalo milk by using serial dilution agar plate technique. A total of twelve isolates were streaked on agar plates on the basis of the colony morphology and color as shown in (Table 5)

Isolation of food spoilage fungi

Identification of fungal isolates

Two fungi were obtained from the stale namkeen.

Fungus 1

From the top of the Petri plate the fungal colony was gray in color surrounded by a white boundary .The fungus contains the basal foot cell, long conidiophores bearing vesicle, one layer of phialids, globose to subglobose conidia. These are the features of *Aspergillus* species (Figure 2).

Fungus 2

From the top of the Petri plate the fungal colony was orange in color from the centre, surrounded by a white boundary The sickle shaped cells were observed under the compound microscope confirming that the fungus obtained belonged to the *Fusarium* species (Figure 3).

Spoiled bread

The culture was powdery in nature. When

Table 1. List of food samples collected for isolation of lactic acid bacteria

Fruit	Milk sample	Ready to eat
Lemon	Buffalo milk	Yakult (probiotic drink) Sporlac

Table 2. List of spoiled food sample used for the isolation of food spoilage fungi

S. No.	Common Name
1	Namkeen (Aloo bhujia -Haldirams)
2	Tomato
3	Bread

Table 3. List of plant products used for the preparation of plant extracts

S. No.	Common name	Botanical name
1	Pomegranate	*Punica* species
2	Turmeric	*Curcuma* species
3	Black pepper	*Pipper* species

Table 4. Antifungal disc used in the study

S. No.	Antifungal disc	Concentration
1	Clotrimazole CC ^{10}	10mcg/disc
2	Ketoconazole KT^{50}	50 mcg/disc
3	Miconazole MIC ^{30}	30 mcg/disc
4	Nystatin NS ^{50}	50 mcg/disc

Table 5. Preliminary identification of lactic acid bacteria

Source	Isolate	Gram Staining	Shape	Endospore Staining	Catalase Test
Lemon	L1	+ ve	Coccus	- ve	+ ve
	L2	+ ve	Coccus	- ve	+ ve
	L3	+ ve	Rods	- ve	- ve
	L4	+ ve	Coccus	- ve	- ve
	L5	+ ve	Short rods	- ve	-ve
	L6	+ ve	Coccobacillus	- ve	- ve
Buffalo Milk	BF1	+ ve	Short rods	- ve	- ve
	BF 2	+ve	Rods	- ve	- ve
	BF 3	+ ve	Coccus	- ve	- ve
Sporlac	SP1	+ ve	Rods	- ve	- ve
Yakult	YK1	+ ve	Rods	- ve	- ve

Table 6. Antifungal activity of lactic acid bacteria against food spoilage fungal isolates

Isolate	*Aspergillus* Species P1	*Fusarium* Species P2	*Penicillium* Species B	*Alternaria* Species T	*Candida* Species C
L1	NA	15	NA	NA	NA
L2	NA	NA	NA	NA	NA
L3	NA	NA	NA	NA	NA
L4	NA	35	NA	NA	NA
L5	NA	NA	NA	NA	NA
L6	16	NA	NA	NA	NA
BF1	NA	NA	NA	NA	NA
BF2	NA	10	NA	NA	NA
BF3	NA	NA	NA	NA	NA
SP1	30	NA	NA	NA	NA
YK1	NA	NA	NA	NA	NA

Table 7. Antifungal activity of pomegranate extracts against food spoilage fungi

Isolate	*Aspergillus* Species P1	*Fusarium* Species P2	*Penicillium* Species B	*Alternaria* Species T	*Candida* Species C
Methanol	32	45	40	NA	NA
Ethanol	NA	NA	NA	NA	NA
Acetone	NA	25	NA	NA	NA

NA-No Activity

Table 8. Antifungal activity of turmeric extracts against food spoilage fungi

Isolate	*Aspergillus* Species P1	*Fusarium* Species P2	*Penicillium* Species B	*Alternaria* Species T	*Candida* Species C
Ethanolic	25	24	NA	NA	NA
Methanolic	25	21	NA	NA	NA
Acetone	NA	NA	NA	NA	NA

NA-No Activity

Table 9. Antifungal activity of black pepper against food spoilage fungi

Isolate	*Aspergillus* Species P1	*Fusarium* Species P2	*Penicillium* Species B	*Alternaria* Species T	*Candida* Species C
Ethanolic	34mm	20mm	NA	NA	15mm
Methanolic	30mm	27mm	NA	NA	18mm
Acetone	NA	NA	NA	NA	NA

NA-No Activity

Table 10. Zone of inhibition (in mm) of antifungal discs against food spoilage fungi

MOLD	Antifungal susceptibility pattern of tested food spoilage fungi (in mm)			
	Clotrimazole CC ^{10}	Ketoconazole KT ^{50}	Miconazole MIC^{30}	Nystatin
Aspergillus from namkeen	15	20	16	15
Fusarium from namkeen	NA	NA	30	NA
Penicillium from bread	20	24	22	NA
Alternaria from tomato	14	16	14	NA
Candida species	10	14	16	NA

NA-No Activity

Table 11. Minimum inhibitory concentration (MIC) of pomegranate methanolic extract against food spoilage fungi using macro dilution agar method

Food spoilage fungi	Minimum inhibitory concentration (MIC) of pomegranate methanolic extract (mg/ml)					
	20	10	5	2.5	1.25	MIC(mg/ml)
Aspergillus species isolated from namkeen	-	-	-	+	+	10
Fusarium species isolated from namkeen	-	-	-	+	+	5
Penicillium species isolated from bread	-	-	+	+	+	10

-No Growth, + Growth, NA-No Activity

observed from the top of the Petri plate the fungal colony was gray to green in color which was surrounded by the thread like yellow periphery. According to the microscopic characteristics the fungus belongs to the *Penicillium* species (Fig. 4).

Spoiled tomato

The culture was somewhat velvet in nature. The fungus was cream in color from the centre and light brown from the middle. The middle light brown portion was surrounded by a cream periphery. Microscopically the fungus belongs to

Table 12. Minimum inhibitory concentration (MIC) of turmeric ethanolic extract against food spoilage fungi using macro dilution agar method

Food spoilage fungi	Minimum inhibitory concentration (MIC) of turmeric ethanolic extract (mg/ml)					
	20	10	5	2.5	1.25	MIC(mg/ml)
Aspergillus species isolated from namkeen	-	+	+	+	+	20
Fusarium species isolated from namkeen	-	+	+	+	+	20

-No Growth, + Growth, NA-No Activity

Table 13. Minimum inhibitory concentration (MIC) of turmeric methanolic extract against food spoilage fungi using macro dilution agar method

Food spoilage fungi	Minimum inhibitory concentration (MIC) of turmeric methanolic extract (mg/ml)					
	20	10	5	2.5	1.25	MIC(mg/ml)
Aspergillus species isolated from namkeen	-	+	+	+	+	20
Fusarium species isolated from namkeen	-	+	+	+	+	20

Table 14. Minimum inhibitory concentration (MIC) of black pepper ethanolic extract against food spoilage fungi using macro dilution agar method

Food spoilage fungi	Minimum inhibitory concentration (MIC) of black pepper ethanolic extract (mg/ml)					
	20	10	5	2.5	1.25	MIC(mg/ml)
Aspergillus species isolated from namkeen	-	-	-	-	-	>1.25
Fusarium species isolated from stale namkeen	-	-	-	-	+	2.5
Candida species	-	-	-	-	+	2.5

Table 15. Minimum inhibitory concentration (MIC) of black pepper methanolic extract against food spoilage fungi using macro dilution agar method

Food spoilage fungi	Minimum inhibitory concentration (MIC) of black pepper methanolic extract (mg/ml)					
	20	10	5	2.5	1.25	MIC(mg/ml)
Aspergillus species isolated from namkeen	-	-	-	-	+	2.5
Fusarium species isolated from stale namkeen	-	-	-	-	+	2.5
Candida species	-	-	-	-	+	2.5

-No Growth, + Growth, NA-No Activity

the *Alternaria* species (Figure 5).

Screening of antifungal activity cell free supernatant obtained from lactic acid bacteria

The cell free supernatant of lactic acid bacteria was screened for their antifungal activity against the isolated food spoilage fungi (standardized to 10^6 cells/ml) by agar well diffusion method (Table 6).

Evaluation of antifungal activity of plant extracts against isolated food spoilage fungi

A total of 3 plant extracts were obtained and these plant extracts were evaluated for their antifungal activity against the isolated food spoilage fungi by agar well diffusion method (Table 7, 8 & 9).

Antifungal susceptibility pattern of tested food spoilage fungi

Four antifungal discs namely Clotrimazole CC ^{10}, Ketoconazole KT ^{50}, Miconazole MIC^{30} and Nystatin NS ^{50} were used to check the antifungal susceptibility pattern of food spoilage fungi (Table 10).

Determination of minimum inhibitory concentration (MIC) of plant extracts against the isolated food spoilage fungi

MIC of methanolic extract of pomegranate against food spoilage fungi

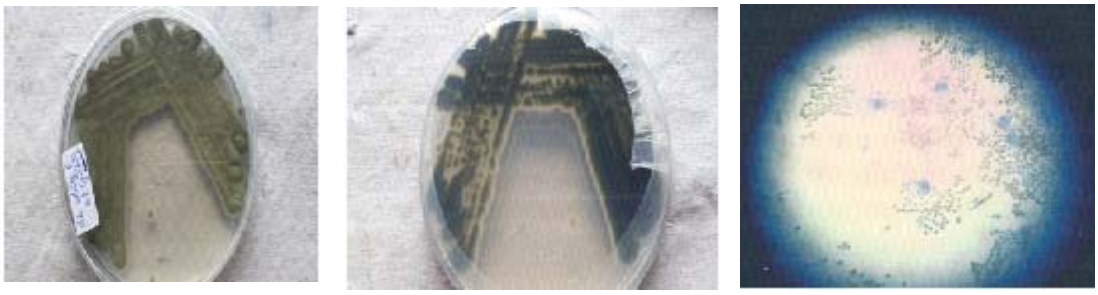
Minimum inhibitory concentration (MIC) of pomegranate methanolic extract was determined against food spoilage fungi using macro dilution

(A) Front View (B) Bottom View (C) Microscopic Features

Fig. 1. Characteristic feature of the *Aspergillus* species isolated from the spoiled namkeen

(A) Front View (B) Bottom View (C) Microscopic Features

Fig. 2. Characteristic features of *Fusarium* species isolated from spoiled Namkeen



(A) Front View (B) Bottom View (C) Microscopic Features

Fig. 3. Characteristic features of *Penicillium* species isolated from spoiled bread

agar method and results obtained (Table 11).

MIC of ethanolic and methanolic extracts of turmeric against food spoilage fungi

Minimum inhibitory concentration (MIC) of turmeric ethanolic and methanolic extracts were determined against food spoilage fungi using macro dilution agar method and results obtained (Table 12 & 13).

MIC of ethanolic and methanolic extracts of black pepper against food spoilage fungi

Minimum inhibitory concentration (MIC) of turmeric ethanolic and methanolic extracts were determined against food spoilage fungi using macro dilution agar method and results obtained (Table 14 & 15).

DISCUSSION

The present research work deals with control of food spoilage fungi by lactic acid bacteria and plant extracts. A total of twelve bacterial isolates were aseptically isolated from four different food samples using serial dilution agar technique. In the present study the approaches in preliminary identification of isolated bacterial strains of morphological characterization like positive growth on MRS Agar medium, negative catalase activity, colony color, positive gram tests and negative endospore test. The food spoilage fungi were isolated from different spoiled foods namely namkeen, bread and tomato. From the namkeen two food spoilage fungi namely, *Aspergillus* species and *Fusarium* species were isolated. The *Pencillium* species were isolated from the bread. The *Alternaria* species was isolated from tomato. The isolated food spoilage fungi were standardized to 10^6 cells/ml and the antifungal activity of the lactic acid bacteria were determined against the food spoilage fungi by using agar well diffusion method.

Out of eleven isolates of lactic acid bacteria, only one isolate L6 isolated from lemon was active against *Aspergillus* species isolated from stale namkeen with a zone of inhibition of 16 mm. The lactic acid bacterial isolate SP1 which were isolated sporlac respectively, were active against *Aspergillus* species isolated had shown a zone of inhibition of 30mm.The lactic acid bacteria isolates had shown good response against the food spoilage fungi isolated from different spoiled food samples. The lactic acid bacterial isolates L4and L6 isolated from lemon were active against the *Fusarium* species isolated from the stale namkeen. The L4 isolate had shown good activity with zone of inhibition of 35 mm .Apart from these lactic acid bacteria isolated from lemon, the isolate L1 isolated from the lemon and BF 2 isolated from the buffalo milk also demonstrated antifungal activity against this fungus with zones of inhibition of 15mm, 10mm respectively. The isolate L6 isolated from lemon had shown the inhibitory effect against the two food spoilage *Aspergillus* species isolated from the stale namkeen and the *Fusarium* species, whereas the maximum zone of inhibition was observed against Fusarium species by the isolate ST4 isolated from lemon. None of the isolates were active against the *Penicillium* species isolated from the spoiled bread and *Alternaria* species isolated from spoiled tomato.

The plant extracts were also used to screen their antifungal activity against the food spoilage fungi. Three plants namely Pomegranate, black pepper and Turmeric were selected for the preparation of plant extracts. The ethanolic and the acetone extract of the pomegranate peel were active against the *Fusarium* species isolated from the stale namkeen with zone of inhibition of 25mm and 20m respectively and no other food spoilage fungi were inhibition by these extracts of the pomegranates peel. Whereas the methanolic extract of the pomegranate peel was active against *Aspergillus* species isolated from the stale namkeen. *Fusarium* and *Penicillium* species with zone of inhibition of 40mm, 32mm, 45mm, and 40mm respectively. The *Alternaria* and *Candida* species was not inhibited by any of the extracts of the pomegranate peel. In the present study, the ethanolic and methanolic extracts of turmeric were prepared and screened for their antifungal activity against the food spoilage fungi from isolated foods. The ethanolic extract of turmeric was active against the *Aspergillus* and *Fusarium* species isolated from the stale namkeen with zone of inhibitions of 28mm and 24mm respectively. The antifungal activity of ethanolic and methanolic extracts of black pepper was screened against the isolated food spoilage fungi as well as the *Candida* species. Both the black pepper extract were active against *Aspergillus* and *Fusarium* species with the zones of inhibition ranging between 20-35mm.

But in the present study the best antifungal activity against food spoilage fungi had been shown by black pepper extracts.

The MIC of the plant extract that had shown antifungal activity against the food spoilage fungi was determined. The MIC of pomegranate methanolic extract was 10 mg/ml against both the *Aspergillus* species and *Penicillium* species. Whereas the MIC of this extract, against Fusarium species was 5 mg/ml and the fungicidal effect was not observed. The MIC of the ethanolic and methanolic extract of turmeric was observed 20mg/ml against the isolated food spoilage fungi.

The MIC of the methanolic extract of black pepper was determined against two food spoilage fungi namely, *Aspergillus* species isolated from stale namkeen and *Fusarium* species isolated from stale namkeen. The MIC of this extract was observed less than 2.5mg/ml against *Aspergillus and Fusarium* species isolated from stale namkeen. The MIC of the ethanolic extract of black pepper was determined against two food spoilage fungi namely *Aspergillus* species isolated from stale namkeen and *Fusarium* species isolated from stale namkeen. The MIC was of this extract was observed less than, 1.25 mg/ml against the *Aspergillus* species and 2.5 mg/ml against *Fusarium* species. The best antifungal effect had shown by black pepper extracts, among the plant extracts used in the current study.

CONCLUSION

The present research work deals with control of food spoilage fungi by lactic acid bacteria and plant extracts. To conclude black pepper extracts had shown the best antifungal effect against the isolated food spoilage fungi in the current study. Henceforth in the future we can use natural/ traditional products such as black pepper for fighting against future food spoilages and fungal contamination caused by *Candida albicans* fungi without having any adverse side effects which are generally seen while using chemical preservatives .

REFERENCES

1. Adams, M.R., Moss, M.Q. Food Microbiology (2nd edition). Panima Publishing Corporation. New Delhi/ Bangalore. 2003; pp. 438.
2. Saranraj, P., Geetha, M. Microbial Spoilage of Bakery Products ans Its Control by Preservatives. *Int. J. Pharm. Biol. Arch,*. 2012; 3(1): 38-48.
3. Tauxe, R.V. Emerging food borne pathogens. *Int. J. Food Microbiol.* 2002; **78**(22): 193-219.
4. Doyle, M.E. Microbial Food Spoilage –Losses and Control Strategies. FRI *Briefings*, 2007; 1-12.
5. Filtenborg, O., Frisvad, J.C., Thrane, U. Moulds in food spoilage. *Int J. Food Microbiol.*, 1996; **33**: 85-102.
6. Dalie, D.K.D., Deschamps, A.M. Richard-Forget, F. Lactic acid bacteri-Potential for control of mould growth and mycotoxins: A review. *Food Control*, 2010; **21**(4): 370-80.
7. Voulgari, K., Hatzikamari, M., Delepoglou, A., Gergakopoulos, P., Litopoulou- Tzanetaki, E., Tazanetakis, N. Antifungal activity of non-starter lactic acid bacteria isolates from dairy products. *Food Control*, 2010; **21**: 136-42.
8. Akpinar- Bayizit, A., Ozcan-Yilmaz, L. Study on the use of yoghurt, whey, lactic acid and starter culture on carrot fermentation. *Polish J Food Nutr. Sci*., 2007; **57**(2): 147-50.
9. Sarnya, S. and Hemashenpagam, N. Antagonistic activity and antibiotic sensitivity of Lactic acid bacteria from fermented dairy products. *Adv. Appl. Sci. Res.*, 2011; **2**(4): 528-34.
10. Rahaman, M.S.A., Thangaraj, S., Salqique, S.M., Khan, K.F. Natheer, S.E. Antimicrobial and Biochemical Analysis of Some Spices Extract against Food Spoilage Pathogens. *Int J Food Saftey*, 2010; **12**: 71-5.
11. Aneja, K. R. Experiments in microbiology, plant pathology and biotechnology (4th edition) New Age International (P) Publishers, New Delhi. 2003; pp. 607.
12. Aneja, K. R., Mehrotra, R.S. Fungal diversity and biotechnology. 1st edition. New Age International (P) Publishers, New Delhi. 2011; pp. 708.
13. Pundir, R. K. and Jain, P. Screening for antifungal activity of commercially available chemical food preservatives. *Int. J. Pharm. Sci. Rev. Res.*, 2010; **5**(2): 25-27
14. Ashmaig, A., Hasan, A. and Gaali, E.E. Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel's milk (Gariss). *Afr. J. Microbiol. Res*., 2009; **3**(8): 451-7.
15. Goyal, R., Dhingra, H., Bajpai, P., Joshi, N. Characterisation of the *Lactobacillus* isolated from different curd samples. *Afr. J. Food Microbiol.*, 2012; **78**(1-2): 79-97.

16. Ibourahema, C., Dauphin, R. D., Destain, J., Philippe, T. Characterization of lactic acid bacteria isolated from poultry farms in Senegal. *Afr. J. Biotechnol*.,. 2008; 7(12): 2006-12.
17. Muhialdin, B. J. and Hassan, J., Screening of lactic acid bactcria for antifungal activity against Aspergillus oryzae. *Am. J. Appl. Sci*., 2011; **8**(5): 447-4514.
18. Shinde, S.L., More, S.M., Junne, S.B. Wadej, S.S. The antifungal activity of five Terminalia species checked by paper disc method. *Int. J. Pharma Res. Dev.*, 2011; **3**: 36-40
19. Dahham, S.S., Ali, M. N., Tabassum, H., Khan, M. Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum Linn*). *American-Eurasian Journal of Agriculture and Environmental Environment*. 2010; **9**(3): 273-81.